

# **Baculoviral Transduction Facilitates TALEN-Mediated Targeted Transgene Integration and Cre/LoxP Cassette Exchange in Human Induced Pluripotent Stem Cells**

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## **SUPPLEMENTARY INFORMATION**

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## Supplementary Table

**Table S1:** Primers used in the current study

Primer Sequence (Forward, FP; Reverse, RP)	Amplicon Size (bp)	Description
FP (AAVS1_SnaBI): 5'-GCGC TACGTA CTGAACCTGAGCCAGCTCCCATA-3' RP (AAVS1_Sall): 5'-ATATGTCGAC GAAGACTAGCTGAGCTCTCGGACC-3'	864	Construct left homologous arm
FP (AAVS1_NotI): 5'-ATATGCGGCCGC CCCTATGTCCACTTCAGGACAGCA-3' RP (AAVS1_BstBI): 5'-GCGC TTCGAATGCCAAGGACTCAAACCCAGAAG-3'	879	Construct right homologous arm
FP (EGFP): 5'-GCGACGTAAACGGCCACAAGTT-3' RP (AAVS1): 5'-GCCTCCCTAACGACCCAGAAGTCCAG-3'	2886	Check for site-specific integration of EGFP donor
FP (WPRE): 5'-GCTACGTCCCTTCGGCCCTCAATC-3' RP (AAVS1): 5'-GCCTCCCTAACGACCCAGAAGTCCAG-3'	3038	Check for site-specific integration of 4F-EGFP donor
FP (EGFP): 5'-GCGACGTAAACGGCCACAAGTT-3' RP (EGFP): 5'-CTGGGTGCTCAGGTAGTGGTTG-3'	554	Design probe for Southern blot
FP (ACTB): 5'-TGGCACCCAGCACAATGAAG-3' RP (ACTB): 5'-GATGGAGGGGCCGGACTC-3'	146	Actin
FP (Oct4): 5'-GAGCAAAACCCGGAGGAGT-3' RP (Oct4): 5' -TTCTCTTCGGGCCTGCAC-3'	310	Oct4 pluripotency marker
FP (Sox2): 5'-GGGAAATGGGAGGGGTGCAAAA-3' RP (Sox2): 5'- TTGCGTGAGTGTGGATGGGATT-3'	151	Sox2 pluripotency marker
FP (Nanog): 5' -GCTTGCCTTGCTTGAAAGCA-3' RP (Nanog): 5' -TTCTTGACCGGGACCTTGTC-3'	256	Nanog pluripotency marker
FP (NELF): 5'-AAGCGTAATTCTGTTGCCTCAAG-3'	196	NELF ectoderm marker

RP (NELF): 5'-GTCCAACCAGTCAAGCTATCATTG-3'		
FP (Pax6): 5'-CAATAATGTTGACGGTGACTATC-3'	179	Pax6 ectoderm marker
RP (Pax6): 5'-AGAAGGAAGCGACACTCTG-3'		
FP ( $\alpha$ -MHC): 5'-GTCATTGCTGAAACCGAGAATG-3'	413	$\alpha$ -MHC mesoderm marker
RP ( $\alpha$ -MHC): 5'-GCAAAGTACTGGATGACACGCT-3'		
FP (PPAR- $\gamma$ ): 5'-ATTGACCCAGAAAGCGATT-3'	154	PPAR- $\gamma$ mesoderm marker
RP (PPAR- $\gamma$ ): 5'-CAAAGGAGTGGGAGTGGTCT-3'		
FP (AL133): 5'-ACTGCTTGTGATGTCTTCTGTG-3'	157	AL133 endoderm marker
RP (AL133): 5'-CGTACACGTCCTCCGAATCC-3'		
FP (AFP): 5'-CCCGAACTTCCAAGGCCATA-3'	101	AFP endoderm marker
RP (AFP): 5'-TACATGGGCCACATCCAGG-3'		
FP (mCherry): 5'-CAACACCGTCTATGGTGAGCAAGGGCGA-3'	3003	Check for site-specific Cre-LoxP cassette exchange of mCherry donor
RP (AAVS1): 5'-GCCTCCCTAACGACCCAGAACGTCCAG-3'		
FP(off-target 1) 5'-TTCTCGTGGTCCCACCAG-3'	337	Off-target site 1
RP(off-target 1) 5'-TGATCTACCTGGGGCTTG-3'		
FP(off-target 2) 5'-AAGAAGACGGTTGGAAAC-3'	440	Off-target site 2
RP(off-target 2) 5'-GTTTATCCTAGGAAGGCAATGTTAT-3'		
FP(off-target 3) 5'-CTTGAGGCTAACGGGTCTG-3'	353	Off-target site 3
RP(off-target 3) 5'-AGAAGGGCTGAGCACTGGTG -3'		
FP(off-target 4) 5'-TGACATCCAATGGCACCCAA -3'	404	Off-target site 4
RP(off-target 4) 5'-CAGAGCACACATTCAAGGGGT -3'		
FP(off-target 5) 5'-AATGAGCTCGTAGACCTGCC -3'	218	Off-target site 5
RP(off-target 5)		

5'- AGTGGCTTCCAAAGATGGAA -3'		
FP(off-target 6) 5'- TGGGAGTAGCATTTCGGCA -3'	335	Off-target site 6
RP(off-target 6) 5'- AGAACGTCCCAGCAAGACAG-3'		
FP(off-target 7) 5'- GCAAGAGCACACTTCCAGC -3'	337	Off-target site 7
RP(off-target 7) 5'- TCTGATGACCCCTCGGTGTA -3'		
FP(off-target 8) 5'- TGAAAGTTGGCCCCAGAGTC-3'	294	Off-target site 8
RP(off-target 8) 5'- ACTAATGGAGCACATGGCCG -3'		
FP(AAVS1) 5'- TCTAACGCTGCCGTGCCGTCTCTCCTGA -3'	1600	Remaining allele deletion
RP(AAVS1) 5'- CGGGGATGCAGGGGACGGGGCTCAGTCTG -3'		

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**Table S2:** Eight BLAST-predicted genomic off-target (OT) sites

Rank	Chr	Location	Sequence	Gap(bp)	Matched bp
AAVS1	19	28116242	CCGAGAGCTCAGCTAGTCTTCTTC TCCAACCCGGGCCCTATGTCC	17	L 15/15; R 15/15
OT1	11	7679322	GCCCTCACCTCAGCCTCCTGGAGGGCCA CCAACCCGGGCCCTACTGTACCA	16	L 10/15; R 13/15
OT2	8	11331521	TCCTCCTTGAGCCAGTCTTCTTCCTCC ATCCCAGGGCCCAGACAGTCA	14	L 7/15; R 10/15
OT3	11	11484751	AGACTGAGCTCAGCTCGTCTTCTTCCTG GACCTTGGACTCCTTGGCAGA	19	L 10/15; R 9/15
OT4	1	4408187	TCCAGGTGCGTGCAGGGACAGGGCTG GGCCCCTGGGGAGGGGCCCTATGTCCA	21	L 7/15; R14/15
OT5	17	26934696	GTGGCAGAACAGACAGCCACTGTTCTGTTGG TCAGCAGGGCATGTGCCTTGT	18	L 8/15; R10/15
OT6	9	37382578	TAAGAATTCTTTCTGGATTGTTACTTT AGTCCCTGGGTGAAAGGAGGA	23	L 6/15; R 6/15
OT7	17	44866370	GCTGAGAACATTCTAGATTGAGAACTAT GTGGAGGTAACACCTGTGCA	26	L 7/15; R 6/15
OT8	1	19097242	TGAGCAGAGCTCAGCTAGTGACCTCT CAGGAAGGCGACGAGGAAGA	19	L 12/15; R 4/15

L: The sequence for left binding domain; R: The sequence for right binding domain.

## Supplementary Figures

**Figure S1.** Single strand annealing (SSA) assay for determination of TALEN cleavage rate in yeast. The assay was performed using custom TALEN service provided by Cellectis Bioresearch (Paris, France). (A) An overview of the SSA assay process. A first haploid strain of yeast expresses a plasmid encoding TALEN sequences while a second haploid strain of yeast expresses a plasmid carrying a non-functional LacZ gene. The LacZ gene is interrupted by an internal duplication of 700 bp to 220 bp while TALEN recognition site is integrated between the two repeats. Upon cleavage with TALEN, the double-strand break is repaired by single-strand annealing. The restoration of a functional LacZ gene allows expression of the  $\beta$ -galactosidase enzyme that can be detected by a blue-white assay. (B) A blue/white colorimetric assay to determine the TALEN cleavage rate in yeast. The  $\beta$ -galactosidase activity is directly associated with the homologous recombination efficiency. The cleavage rate of the TALENs detected in the assay was 87%.

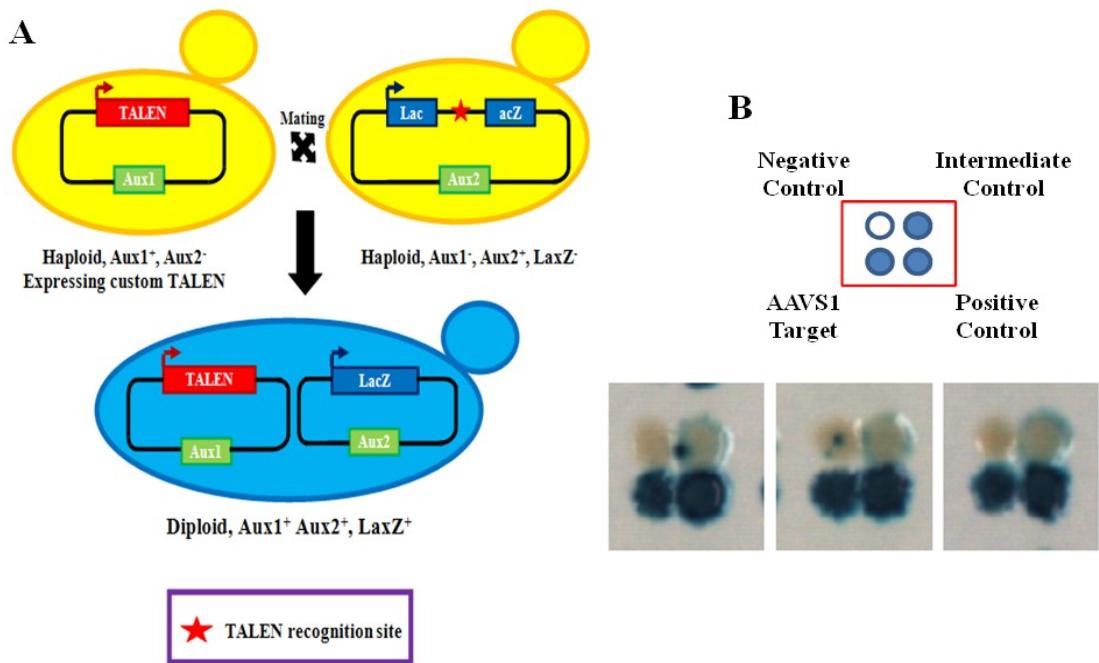
**Figure S2.** PCR genotyping to confirm TALEN-mediated, AAVS1-specific transgene integration in human U87 cells. A primer specific for the eGFP gene present in the DNA donor BV-eGFP and a primer specific for chromosome 19 downstream of the 3' end of the right homologous arm (see Fig. 1) were used. The amplification of a 2.9 kb fragment demonstrates the successful AAVS1 modification through BV-TALEN mediated HR. Including results in Fig. 2C, totally 41 clones were examined.

**Figure S3.** Southern blot analysis to detect the modified AAVS1. Five transgenic clones were examined after digestion with ApaLI and hybridization. Single 12 kb fragment (arrow) was detected in these clones. Including results in Fig. 2D, totally 8 clones were examined.

**Figure S4.** Analysis of NHEJ mutations in U87 cells. The genomic off-target sites were amplified from genomic DNA isolated from wild-type (WT) U87 and three AAVS1-targeted clones generated in this study. DNA sequencing was performed to examine NHEJ mutations at eight BLAST-predicted genomic off-target (OT) sites (Table S2). Chromatograph results are presented. The reference sequences for each OT site are listed on the top.

**Figure S5.** Karyotype diagram of transgenic EGFP-positive human iPSCs. Homologous pairs of chromosomes are identified by their general shape, length, and the pattern of banding produced by a Giemsa staining. Results from 6 iPSCs are shown.

Figure S1



**Figure S2**

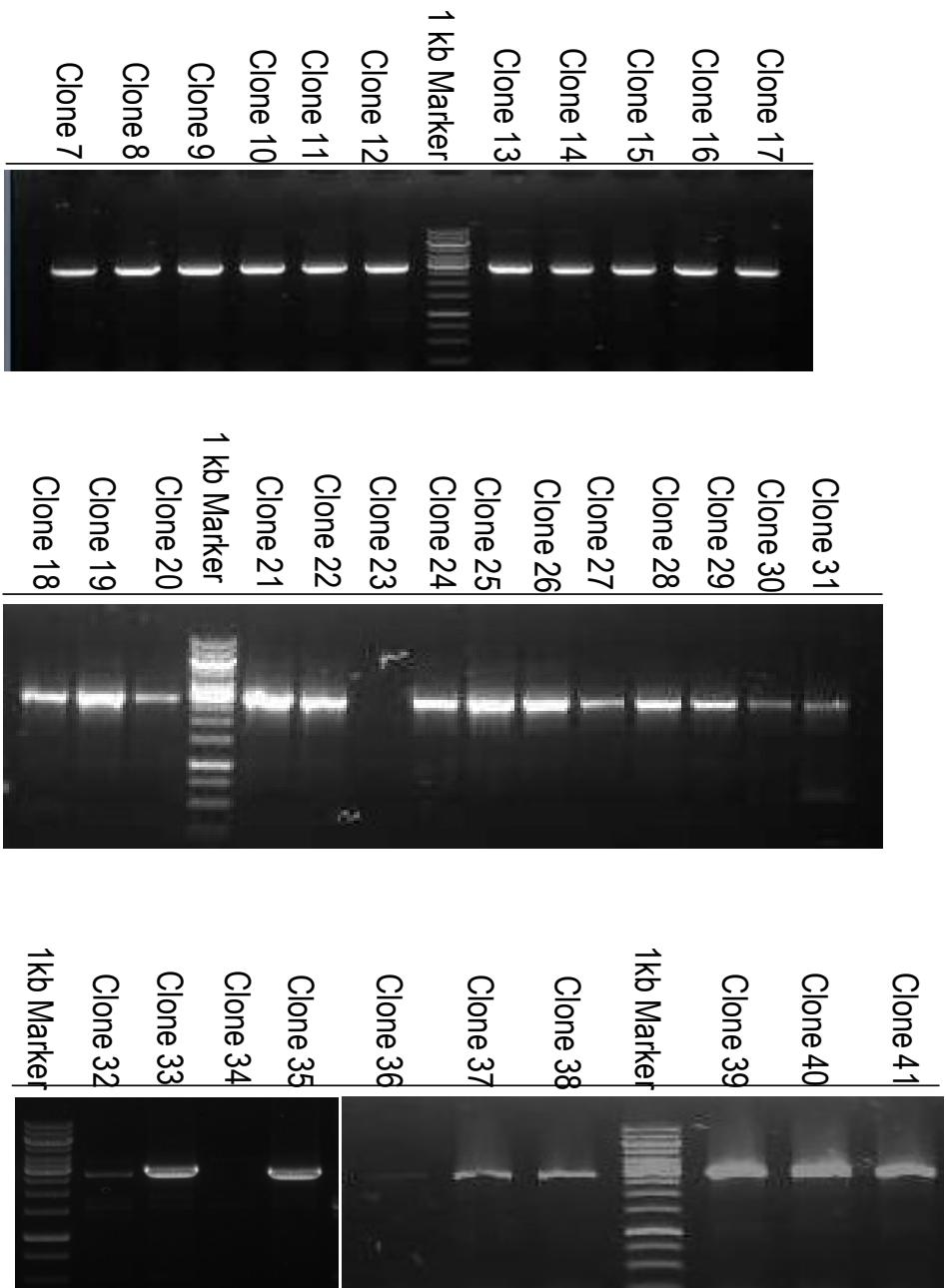


Figure S3

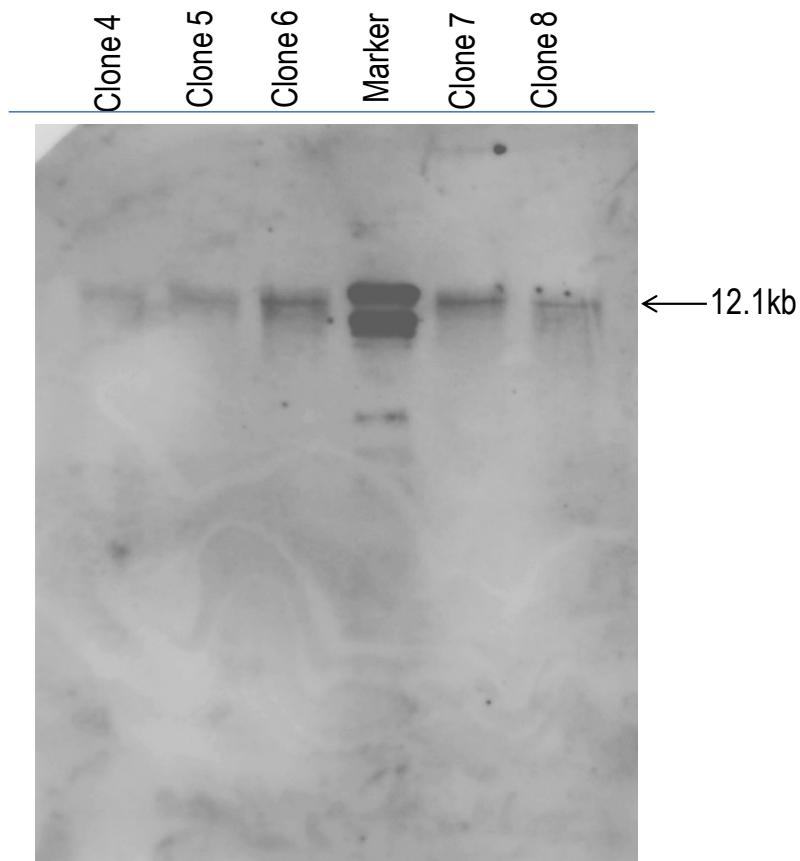


Figure S4

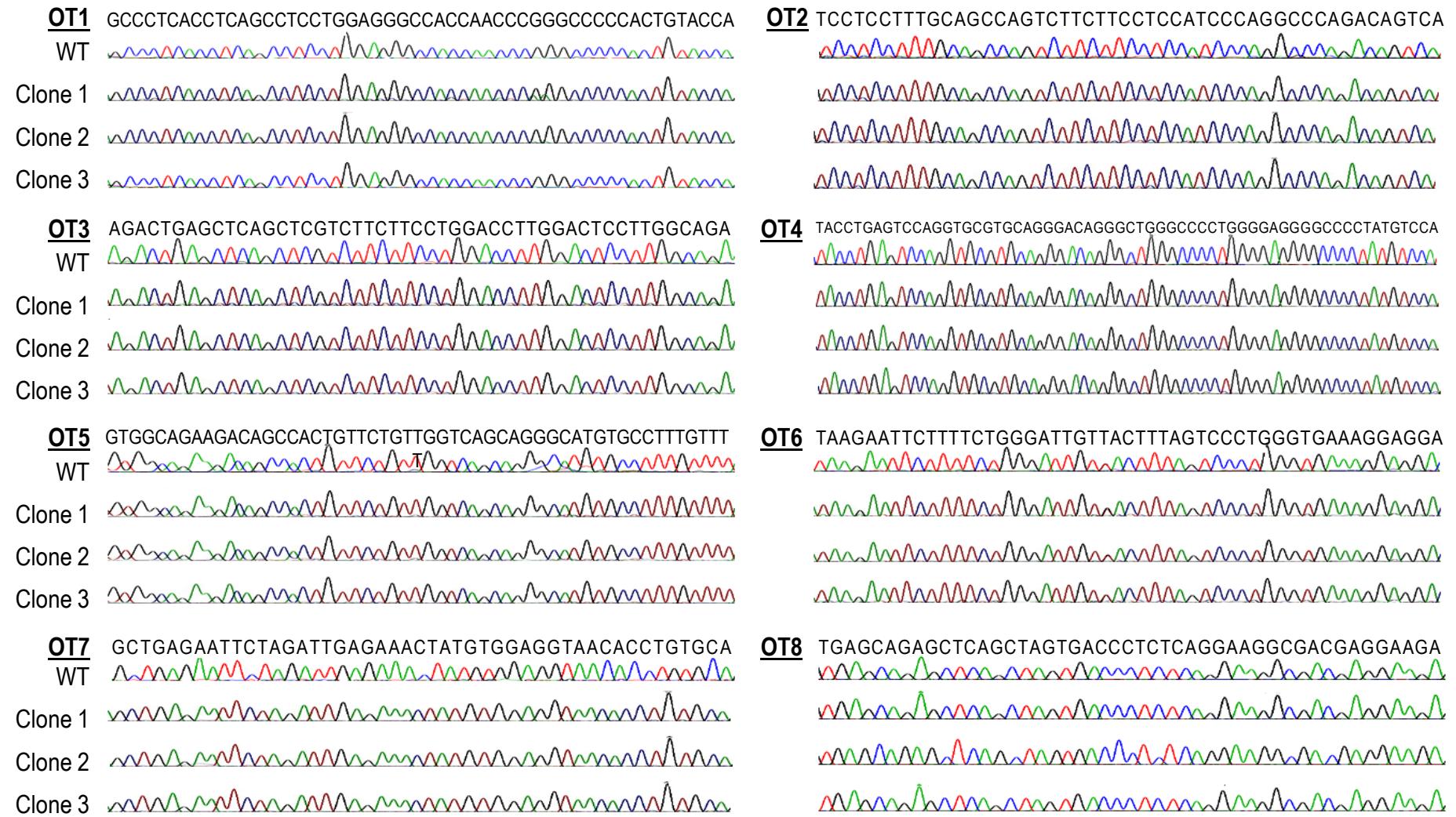
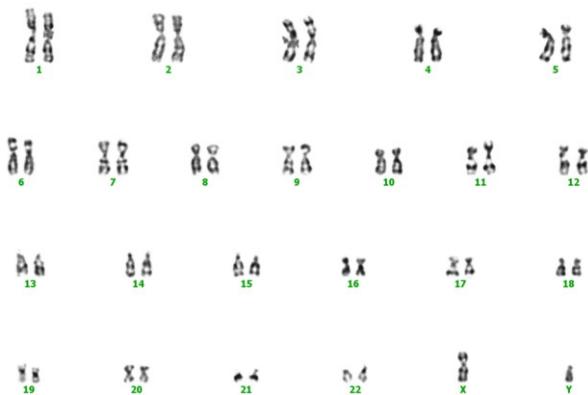


Figure S5

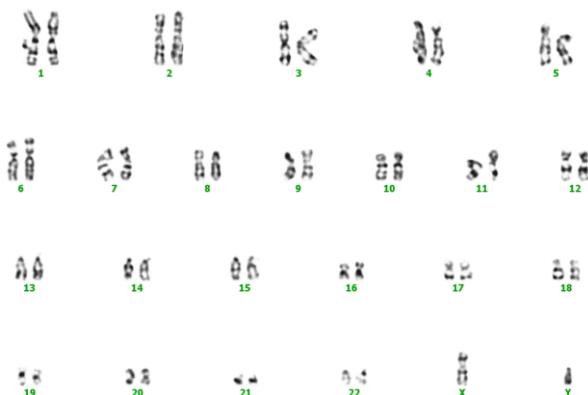
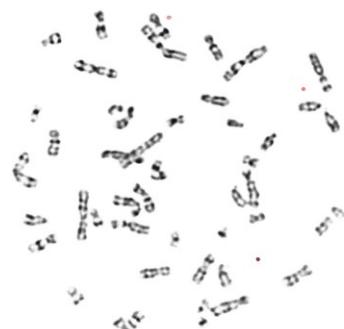
iPSC #1



iPSC #2



iPSC #3

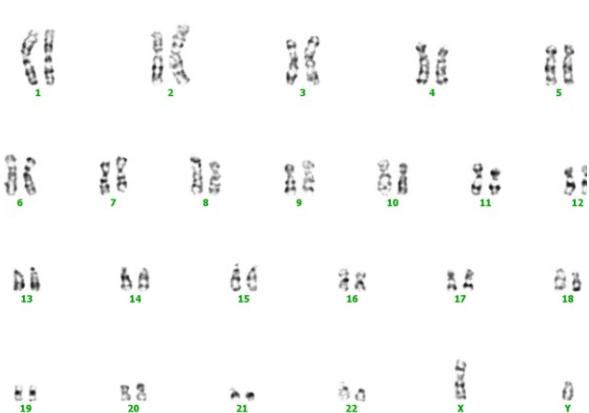


**Figure S5**

iPSC #4



iPSC #5



iPSC #6

